短 報

Bo FAN[a], Masaki BABA[a], Atsuo MIZUNO[a], Yoshihito OKADA[a], Jingda XU[b] and Toru OKUYAMA[a]: **Studies on the Chemical Consitituents of *Peucedanum japonicum* (Umbelliferae)**

ボタンボウフウ（セリ科）の化学成分（范 波[a]，馬場正樹[a]，水野敦夫[a]，岡田嘉仁[a]，徐景達[b]，奥山 徹[a]）

In connection with our previous studies on the plants of the genus of *Peucedanum* (Okuyama et al. 1981, 1986), we have investigated *P. japonicum* Thunb., which is a perennial herb distributed in Japan, Korea, Taiwan, Mainland China and the Philippines (Ono et al. 1996). This plant has been used as a folk medicine to treat cold and cough in Japan and Taiwan, and to treat some intestinal diseases in Indonesia (Mitsuhashi 1988). Some coumarins were reported to be isolated from this plant (Chen et al. 1996, Ikeshiro et al. 1993). This paper describes the isolation and spectral data of eleven coumarins, two saccharides and stigmasterol. This is the first report on isolation of dihydrosamidin (2) from the aerial part and (+)-3'(R),4'(R)-3'-acetoxy-4'-senecioyloxy-3,4'-dihydroseselin (7), umbelliferone (11) and stigmasterol from the root of this plant. Melting points were determined with a Yanagimoto micromelting point apparatus and were uncorrected. EI-MS spectra were obtained by a JMS-DX302 mass spectrometer. Optical rotation was measured on JASCO DIP-370. Chromatographic separations were carried out on Silica gel 60 (Kanto, Chemical Co., Inc.) open column, HPLC packed column of COSMOSIL (No. 750-00, size: 20×250 mm), ODS open column (COSMOIL 75 C18-OPN) and ODS column for HPLC (Senshu Pak PECASIL, size: 10×250 mm). TLC (Silica gel 60 F_{254} Merck) was detected under UV at 365 nm and 254 nm and spraying with sulfuric acid.

Isolation procedure

The dried ground aerial part (2.6 kg) of *Peucedamum japonicum* was extracted twice with AcOEt under reflux for 3 hours. The solution was concentrated in vacuo at 40℃ to yield an extract (60 g). The extract was subjected to column chromatography on silica gel, eluting the column initially with *n*-hexane-AcOEt (3:1), followed by stepwise increments of polarity by gradual addition of AcOEt to afford four fractions (A1–A4). Fraction A2 was separated on silica gel column using *n*-hexane-AcOEt (3:1) as eluting solvent, and gradually increasing the polarity with AcOEt to afford three fractions (B1–B 3). Fraction B2 was further purified by HPLC eluting with *n*-hexane-AcOEt (3:2) to give compound **1** (302 mg). The fraction A3 was rechromatographed on silica gel CC using *n*-hexane-AcOEt (6:1) as eluting solvent to afford five fractions (C1–C5). Fraction C2 was further purified on HPLC eluting with *n*-hexane-AcOEt (3:2) to yield compound **2** (176 mg), compound **3** (80 mg), compound **4** (333 mg), and compound **1** (753 mg), respectively. Fraction C3 was further separated over HPLC under the same condition as fraction C2 to give compound **4** (152 mg) and compound **1** (1.1 g). Fraction C4 was purified to yield compound **1** (793 mg).

The dried and chopped root of P. japonicum (4.5 kg) was extracted twice with AcOEt under reflux for 3 hours. After filtration, the solution was left at room temperature for two days to yield AcOEt solution and crystalline substance which was recrystallized with MeOH to afford mannitol and sucrose. The AcOEt solution was evaporated under reduced pressure to give an extract (150 g). Eighty g of the extract was

chromatographed on silica gel with *n*-hexane-AcOEt (100:1), followed by increasing polarity of eluting solvent by adding AcOEt gradually to AcOEt 100%. Fraction 1 (21.1 g), fraction 2 (2.6 g), fraction 3 (22.2 g), fraction 4 (20.8 g) and fraction 5 (2.9 g) were gained. Fraction 2 (2.6 g) was recrystallized from MeOH several times to give stigmasterol (83 mg). Two g of fraction 3 was subjected to ODS open column, eluting with MeOH-H_2O (4:1) to give three fractions D1, D2 and D3. D1 was separated on ODS HPLC, eluting with MeOH-H_2O (4:1) to yield compound **10** (7.6 mg). D2 was purified on HPLC with *n*-hexane-AcOEt (2:1) to give compound **7** (260 mg). D3 was rechromatographed on HPLC with *n*-hexane-AcOEt (3:1) to yield compound **5** (31 mg) and compound **3** (116 mg). Two g of fraction 4 was subjected on ODS open column with MeOH-H_2O (4:1) to give three fractions E1, E2 and E3. E1 was recrystallized with MeOH several times to yield stigmasterol (15 mg). E2 was separated on HPLC with *n*-hexane-AcOEt (2:1) to give compound **8** (35 mg), compound **7** (693 mg), compound **6** (11 mg) and fraction F. Fraction F was further purified on HPLC, eluting with *n*-hexane-AcOEt (3:1) to yield compound **9** (9 mg). Fraction 5 was rechromatographed on silica gel with a gradient mixture of *n*-hexane-AcOEt (1:1→100% AcOEt) to yield compound **11** (20 mg).

(+)-Samidin (1) white powder, mp. 135.0–138.0℃; $[\alpha]_D$+13.8° (EtOH, c0.10, 30℃); EI-MS: 386 [M^+], 287, 244, 229; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.22 (1H, d, J = 9.6 Hz, H-3), 7.59 (1H, d, J = 9.6 Hz, H-4), 7.35 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 5.30 (1H, d, J = 5.0 Hz, H-3'), 6.59 (1H, d, J = 5.0 Hz, H-4'), 1.46 (3H, s, gem-Me), 1.42 (3H, s, gem-Me), 5.64 (1H, br s), 2.23 (3H, d, J = 1.3 Hz, Me), 1.89 (3H, d, J = 1.3 Hz, Me), 2.09 (3H, s, OCOMe).

Dihydrosamidin (**2**) white powder, mp. 116.0–118.0℃; $[\alpha]_D$+4.0° (EtOH, c0.10, 30 ℃): EI-MS: 388 [M^+], 287, 261, 244, 229; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.23 (1H, d, J = 9.6 Hz, H-3), 7.61 (1H, d, J = 9.6 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 5.31 (1H, d, J = 5.0 Hz, H-3'), 6.55 (1H, d, J = 5.0 Hz, H-4'), 1.45 (3H, s, gem-Me), 1.42 (3H, s, gem-Me), 2.33–2.24 (2H, m), 2.20–2.17 (1H, m), 1.00 (3H, d, J = 4.0 Hz, Me), 0.98 (3H, d, J = 4.0 Hz, Me), 2.10 (3H, s, OCOMe).

cis -3',4'-Disenecioylkhellactone (**3**) white powder, mp. 112.0–115.0℃; $[\alpha]_D$ -23.0° ($CHCl_3$, c0.10, 30 ℃): EI-MS: 426 [M^+], 326, 311, 244, 213, 83, 55; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.20 (1H, d, J = 9.6 Hz, H-3), 7.58 (1H, d, J = 9.6 Hz, H-4), 7.34 (1H, d, J = 8.6 Hz, H-5), 5.35 (1H, d, J = 8.6 Hz, H-6), 5.35 (1H, d, J = 5.0 Hz, H-3'), 6.62 (1H, d, J = 5.0 Hz, H-4'), 1.46 (3H, s, gem-Me), 1.42 (3H, s, gem-Me), 5.66 (1H, br s), 5.62 (1H, br s), 2.19 (3H, d, J = 1.3 Hz, Me), 2.15 (3H, d, J = 1.3 Hz, Me), 1.89 (3H, d, J = 1.3 Hz, Me), 1.88 (3H, d, J = 1.3 Hz, Me).

(+)-Praeruptorin A (**4**) white powder, mp. 131.0–133.0℃; $[\alpha]_D$ +52.0° ($CHCl_3$, c0.10, 30℃): EI-MS: 386 [M^+], 286, 244, 229, 200, 100; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.24 (1H, d, J = 9.6 Hz, H-3), 7.61 (1H, d, J = 9.6 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-5), 6.81 (1H, d, J = 8.6 Hz, H-6), 5.41 (1H, d, J = 5.0 Hz, H-3'), 6.60 (1H, d, J = 5.0 Hz, H-4'), 1.48 (3H, s, gem-Me), 1.44 (3H, s, gem-Me), 6.18–6.09 (1H, m), 1.96 (3H, br s, Me), 1.88 (3H, br s, Me), 2.11 (3H, s, OCOMe).

(+)-Anomalin (**5**) amorphous, $[\alpha]_D$+22.0° ($CHCl_3$, c0.134, 32℃): EI-MS: 426 [M^+], 327, 326, 311, 261, 244, 229, 227, 213, 83, 55; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.21 (1H, d, J = 9.6 Hz, H-3), 7.58 (1H, d, J = 9.6 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-5), 6.81 (1H, d, J = 8.6 Hz, H-6), 5.45 (1H, d, J = 5.0 Hz, H-3'), 6.71 (1H, d, J = 5.0 Hz, H-4'), 1.49 (3H, s, gem-Me), 1.46 (3H, s, gem-Me), 6.12 (1H, br d, J = 7.3 Hz), 6.02 (1H, br d, J = 7.3 Hz), 1.97 (6H, m, 2 ×Me), 1.85 (3H, m, Me), 1.83 (3H, m, Me).

Peujaponisinol A (**6**) amorphous, $[\alpha]_D$ −2.2° ($CHCl_3$, c0.104, 32℃): EI-MS: 344 [M^+], 261, 244, 229, 191, 83, 55; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.25 (1H, d, J = 9.6 Hz, H-3), 7.64 (1H, d, J = 9.6 Hz, H-4), 7.33 (1H, d, J = 8.6 Hz, H-5), 6.79 (1H, d, J = 8.6 Hz, H-6), 5.21 (1H, d, J = 5.0 Hz, H-3'), 5.43 (1H, d, J = 5.0 Hz, H-4'), 1.49 (3H, s, gem-Me), 1.41 (3H, s, gem-Me), 5.80 (1H, s), 2.20 (3H, d, J = 1.0 Hz, Me), 1.92 (3H, d, J = 1.0 Hz, Me), 3.24 (1H, br s.-OH).

(+)-3'(*R*),4'(*R*)-3'-Acetoxy-4'-senecioyloxy-3',4'-dihydroseselin (**7**) amorphous, $[\alpha]_D$ +10.7° (EtOH, c0.118, 33℃): EI-MS: 386 [M^+], 326, 311, 287, 261, 244, 299, 83; ^{1}H NMR (in $CDCl_3$) δ (ppm): 6.21 (1H, d, J = 9.6 Hz, H-3), 7.61 (1H, d, J = 9.6 Hz, H-4), 7.38 (1H, d, J = 8.6 Hz, H-5), 6.81 (1H, d, J = 8.6 Hz, H-6), 5.29 (1H, d, J = 4.9 Hz, H-3'), 6.58 (1H, d, J = 4.9 Hz, H-4'), 1.47 (3H, s, gem-Me), 1.43 (3H, s, gem-Me), 2.09 (3H, s, OCOMe), 5.64 (1H, s), 2.23

(3H, s, Me), 1.90 (3H, s, Me).

Pteryxin (**8**) amorphous, $[\alpha]_D$ +20.7° ($CHCl_3$, c0.082, 32℃): EI-MS: 386 [M^+], 326, 311, 287, 261, 245, 229, 213, 191, 83, 55; ^1H NMR (in $CDCl_3$) δ (ppm): 6.20 (1H, d, J = 9.6 Hz, H-3), 7.60 (1H, d, J = 9.6 Hz, H-4), 7.37 (1H, d, J = 8.6 Hz, H-5), 6.81 (1H, d, J = 8.6 Hz, H-6), 5.35 (1H, d, J = 5.0 Hz, H-3'), 6.63 (1H, d, J = 5.0 Hz, H-4'), 1.46 (3H, s, gem-Me), 1.43 (3H, s, gem-Me), 2.09 (3H, s, OCOMe), 6.05 (1H, m), 2.00 (3H, dd, J = 7.4, 1.2 Hz, Me), 1.87 (3H, d, J = 1.3 Hz, Me).

(–)-*trans*-3'-Acetyl-4'-senecioylkhellactone (**9**) amorphous, $[\alpha]_D$ −3.6° ($CHCl_3$, c0.14, 32℃): EI-MS: 386 [M^+], 326, 311, 287, 261, 245, 229, 83, 55, 43; ^1H NMR (in $CDCl_3$) δ (ppm): 6.23 (1H, d, J = 9.6 Hz, H-3), 7.60 (1H, d, J = 9.6 Hz, H-4), 7.36 (1H, d, J = 8.6 Hz, H-5), 6.82 (1H, d, J = 8.6 Hz, H-6), 5.32 (1H, d, J = 3.6 Hz, H-3'), 6.20 (1H, d, J = 3.6 Hz, H-4'), 1.46 (3H, s, gem-Me), 1.38 (3H, s, gem-Me), 2.09 (3H, s, OCOMe), 5.62 (1H, m), 2.24 (3H, d, J = 1.2 Hz, Me), 1.87 (3H, d, J = 1.2 Hz, Me).

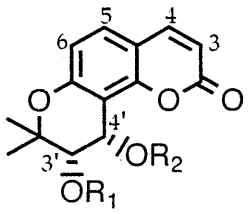
Results and Discussion

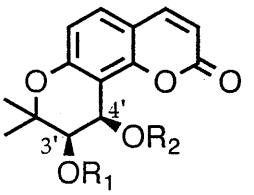
Many pyranocoumarins and furocoumarins from Chinese crude drug "Qian-Hu" (*P. praeruptorum* and *P. decursivum*) were isolated (Okuyama et al. 1981, 1986). This time we obtained nine pyranocoumarins from *P. japonicum*. Furthremore some pyranocoumarins such as (+) praeruptorin A, (+) anomalin, etc. were gained from both *P. praeruptorum* and *P. japonicum*. These suggested that pyranocoumarins were common compounds in some Peucedanum genus plants. And it was reasonable to consider there were some links between antitumor activity and some pyranocoumarins (Nishino et al. 1987, 1990). That is one of the reasons why we have studied *Peucedanum* genus plants.

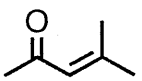
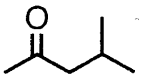
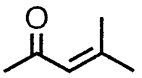
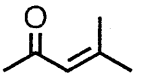
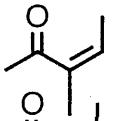
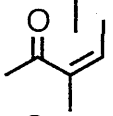
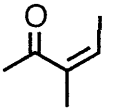
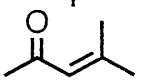
Table 1. ^{13}C NMR spectral data of **1–9** (ppm from TMS, in $CDCl_3$)

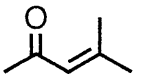
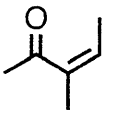
C	1	2	3	4	5	6	7	8	9
2	159.8	159.8	159.9	159.9	159.7	160.6	159.7	159.7	159.9
3	113.3	113.3	113.2	113.2	113.3	112.5	113.0	113.3	113.2
4	143.1	143.2	143.2	143.3	143.2	143.9	143.1	143.1	143.2
5	129.1	129.3	129.0	129.1	129.2	128.7	129.1	129.2	129.0
6	114.3	114.3	114.4	114.3	114.4	114.6	114.3	114.4	114.4
7	156.6	156.6	156.8	156.7	156.8	156.1	156.5	156.6	156.6
8	107.5	107.2	107.6	107.1	107.6	110.7	107.2	107.3	106.8
4a	112.5	112.5	112.5	112.5	112.5	112.4	112.4	112.5	112.5
8a	154.0	154.0	154.1	154.0	154.2	154.4	153.8	154.0	154.2
2'	77.2	77.2	77.2	77.2	77.5	77.8	76.5	77.2	77.2
3'	70.6	70.6	69.4	69.8	70.3	71.4	70.6	70.2	71.4
4'	59.6	60.4	59.8	61.0	60.2	60.2	59.4	60.1	62.4
gem-Me									
	25.3	25.4	25.1	24.9	25.5	25.5	25.3	25.3	23.8
	22.2	22.2	22.7	23.0	22.8	22.6	22.0	20.3	23.7
others									
	R^1	R^1	R^1/R^2	R^1	R^1/R^2	R^1	R^1	R^1	R^1
	165.2	172.0	165.2	166.5	166.5	165.6	169.8	169.8	169.4
	158.2	43.3	165.1	139.8	166.3	159.2	20.0	22.1	20.7
	114.7	25.5	158.2	127.0	139.8	115.1			
	27.4	22.5	157.6	20.5	138.4	27.5	R^2	R^2	R^2
	20.4	22.4	115.3	15.8	127.4	20.5	165.1	166.8	164.7
			115.2		127.1		158.0	137.7	158.9
	R^2	R^2	27.5	R^2	20.43		114.9	127.5	114.9
	169.9	169.8	27.4	169.8	20.38		27.3	20.7	27.5
	20.7	20.7	20.4	20.7	15.8		20.4	15.5	20.5
			20.3		15.6				

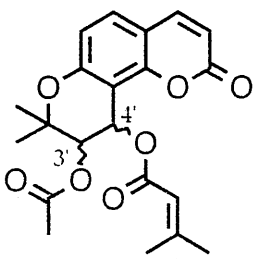
The ^{1}H NMR spectrum of compound 1 to 9 indicated that they were khellactone type coumarins, with the characteristic three pairs of doublet H-3 and H-4 (J = 9.6 Hz), H-5 and H-6 (J = 8.6 Hz), H-3' and H-4' (J = 5.0 Hz to *cis*-form and 3.5 Hz to *trans*-form, respectively (Okuyama et al. 1981)) and geminal methyl singlets ($\Delta\ \delta$ = 0.03 to *cis*-form except **6** or 0.08 to *trans*-form (Gonzalez et al. 1979)). The ^{13}C NMR spectra of these compounds corroborated the presence of pyranocoumarin, exhibiting sig-



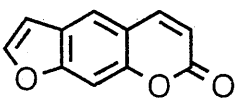


	R1	R2
1		-COCH3
2		-COCH3
3		
4		-COCH3
5		
6		H

	R1	R2
7	-COCH3	
8	-COCH3	



9



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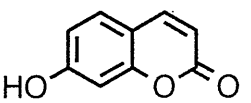

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Fig. 1. Structures of the compounds from *Peucedanum japonicum*.

nals for a coumarin skeleton (eight aromatic carbons and one ester carbonyl), along with gem-dimethyls and heteroatom ring consisting of one quaternary carbon (δ was about 77 ppm) and two methines (one's shift was about 70 ppm, another was about 60 ppm). The coupling constants $J_{3',4'}$ and the $\Delta\ \delta$ values of geminal methyls suggested that the compound **1–8** were *cis*-forms, whereas coupound **9** was *trans*-form. The EI mass spectrum and HMBC of **7** indicated the acetoxy group at C-3' and senecioyloxy group and angeloyloxy group at C-4' in **7** and **8**, respectively (Shaath et al. 1976). HMBC of **1**, **2**, **4**, suggested acetoxy group at C-4' and senecioyloxy, isovaleryloxy and angeloyloxy group at C-3' in **1**, **2**, **4**, respectively. The EI mass spectrum of **9** indicated an acetoxy group at C-3' and senecioyloxy group at C-4' (Shaath et al. 1976). The absolute configurations of **1** to **8** were determined by their optical rotation $[\alpha]_D$. However, the absolute configuration of **9** could not be determined yet, because neither its $[\alpha]_D$ nor its chiral isomer's $[\alpha]_D$ were reported. Further study is necessary to determine its absolute configuration by alkaline hydrolysis on this compound.

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ボタンボウフウは日本，朝鮮半島，台湾，中国及びフィリピンなどに分布し，沖縄では長命草という名前で市場で売られている．民間では鎮咳，利尿，強壮作用があるとして，感冒，滋養強壮に使用されている．台湾ではインフルエンザの治療に，インドネシアでは腸の疾患の治療に用いられている．成分研究としては，主に coumarin 類の単離が報告され，薬理活性としては細胞毒性活性の検討，血小板凝集抑制作用の検討などがある．今回11種の coumarin，2種の saccharide と stigmasterol をボタンボウフウより単離した．ボタンボウフウの発がんプロモーター抑制作用を現在検討中である．

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